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INTERNATIONAL APPLICATION PUBLISHED UNDER THE ~~PATENT~~ COOPERATION ~~TREATY (PCT)~~

(51) International Patent Classification 6 : C12N 15/12, C07K 14/47

A2

(11) International Publication Number: WO 99/06554

(43) International Publication Date: 11 February 1999 (11.02.99)

(21) International Application Number: PCT/IB98/01238

(22) International Filing Date: 31 July 1998 (31.07.98)

(30) Priority Data:
08/905,134 1 August 1997 (01.08.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: 5' ESTs FOR SECRETED PROTEINS EXPRESSED IN MUSCLE AND OTHER MESODERMAL TISSUES

(57) Abstract

The sequences of 5' ESTs derived from mRNAs encoding secreted proteins are disclosed. The 5' ESTs may be to obtain cDNAs and genomic DNAs corresponding to the 5' ESTs. The 5' ESTs may also be used in diagnostic, forensic, gene therapy, and chromosome mapping procedures. Upstream regulatory sequences may also be obtained using the 5' ESTs. The 5' ESTs may also be used to design expression vectors and secretion vectors.

providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein. Alternatively, as described in more detail below, genes encoding proteins involved in any of the above mentioned activities or nucleic acids regulating the expression of such proteins may be introduced into appropriate host cells to increase or decrease the expression of the proteins as desired.

EXAMPLE 42

Identification of Proteins which Interact with Polypeptides Encoded by Extended cDNAs

Proteins which interact with the polypeptides encoded by cDNAs derived from the 5' ESTs or fragments thereof, such as receptor proteins, may be identified using two hybrid systems such as the Matchmaker Two Hybrid System 2 (Catalog No. K1604-1, Clontech). As described in the manual accompanying the kit which is incorporated herein by reference, the the cDNAs derived from 5' ESTs, or fragments thereof, are inserted into an expression vector such that they are in frame with DNA encoding the DNA binding domain of the yeast transcriptional activator GAL4. cDNAs in a cDNA library which encode proteins which might interact with the polypeptides encoded by the extended cDNAs or portions thereof are inserted into a second expression vector such that they are in frame with DNA encoding the activation domain of GAL4. The two expression plasmids are transformed into yeast and the yeast are plated on selection medium which selects for expression of selectable markers on each of the expression vectors as well as GAL4 dependent expression of the HIS3 gene. Transformants capable of growing on medium lacking histidine are screened for GAL4 dependent lacZ expression. Those cells which are positive in both the histidine selection and the lacZ assay contain plasmids encoding proteins which interact with the polypeptide encoded by the extended cDNAs or portions thereof.

Alternatively, the system described in Lustig *et al.*, *Methods in Enzymology* **283**: 83-99, 1997, and in U.S. Patent No. 5,654,150, the disclosure of which is incorporated herein by reference, may be used for identifying molecules which interact with the polypeptides encoded by extended cDNAs. In such systems, *in vitro* transcription reactions are performed on a pool of vectors containing extended cDNA inserts cloned downstream of a promoter which drives *in vitro* transcription. The resulting pools of mRNAs are introduced into *Xenopus laevis* oocytes. The oocytes are then assayed for a desired activity.

Alternatively, the pooled *in vitro* transcription products produced as described above may be translated *in vitro*. The pooled *in vitro* translation products can be assayed for a desired activity or for interaction with a known polypeptide.

Proteins or other molecules interacting with polypeptides encoded by extended cDNAs can be found by a variety of additional techniques. In one method, affinity columns containing the polypeptide encoded by the extended cDNA or a portion thereof can be constructed. In some versions, of this method the affinity column contains chimeric proteins in which the protein encoded by the extended cDNA or a portion thereof is fused to glutathione S-transferase. A mixture of cellular proteins or pool of expressed proteins as described above and is applied to the affinity column. Proteins interacting with the polypeptide attached to the column can then be isolated and analyzed on 2-D electrophoresis gel as described in Ramunsen *et al.*, *Electrophoresis* **18**:588-598, 1997, the disclosure of which is incorporated herein by reference. Alternatively, the proteins retained on the affinity column can be purified by electrophoresis based methods and sequenced. The same method can be used to isolate antibodies, to screen phage display products, or to screen phage display human antibodies.

Proteins interacting with polypeptides encoded by extended cDNAs or portions thereof can also be screened by using an Optical Biosensor as described in Edwards and Leatherbarrow, *Analytical Biochemistry* **246**:1-6, 1997, the disclosure of which is incorporated herein by reference. The main advantage of the method is that it allows the determination of the association rate between the protein and other interacting molecules. Thus, it is possible to specifically select interacting molecules with a high or low association rate. Typically a target molecule is linked to the sensor surface (through a carboxymethl dextran matrix) and a sample of test molecules is placed in contact with

least 15 amino acids of the proteins encoded by the above cDNAs. In other embodiments, the antibodies may be capable of binding fragments of at least 25 amino acids of the proteins expressed from the extended cDNAs which comprise at least 25 amino acids of the proteins encoded by the above cDNAs. In further embodiments, the antibodies may be capable of binding fragments of at least 40 amino acids of the proteins encoded by the above cDNAs.

EXAMPLE 43

Production of an Antibody to a Human Protein

Substantially pure protein or polypeptide is isolated from the transfected or transformed cells as described in Example 30. The concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few μg/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

1. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, and Milstein, *Nature* 256:495, 1975 or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, *Meth. Enzymol.* 70:419, 1980, the disclosure of which is incorporated herein by reference and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis *et al.* in *Basic Methods in Molecular Biology*

V. Use of 5' ESTs or Sequences Obtainable Therefrom or Portions Thereof as Reagents

The 5' ESTs of the present invention (or cDNAs or genomic DNAs obtainable therefrom) may be used as reagents in isolation procedures, diagnostic assays, and forensic procedures. For example, sequences from the 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom) may be detectably labeled and used as probes to isolate other sequences capable of hybridizing to them. In addition, sequences from 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom) may be used to design PCR primers to be used in isolation, diagnostic, or forensic procedures.

1. Use of 5' ESTs or Sequences Obtainable Therefrom or Portions Thereof in Isolation, Diagnostic and Forensic Procedures

EXAMPLE 44

Preparation of PCR Primers and Amplification of DNA

The 5' EST sequences (or cDNAs or genomic DNAs obtainable therefrom) may be used to prepare PCR primers for a variety of applications, including isolation procedures for cloning nucleic acids capable of hybridizing to such sequences, diagnostic techniques and forensic techniques. The PCR primers are at least 10 bases, and preferably at least 12, 15, or 17 bases in length. More preferably, the PCR primers are at least 20-30 bases in length. In some embodiments, the PCR primers may be more than 30 bases in length. It is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see Molecular Cloning to Genetic Engineering, White Ed. *in Methods in Molecular Biology* 67: Humana Press, Totowa 1997, the disclosure of which is incorporated herein by reference. In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation,

hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites.

EXAMPLE 45

Use of 5'ESTs as Probes

Probes derived from 5' ESTs (or cDNAs or genomic DNAs obtainable therefrom), including full length cDNAs or genomic sequences, may be labeled with detectable labels familiar to those skilled in the art, including radioisotopes and non-radioactive labels, to provide a detectable probe. The detectable probe may be single stranded or double stranded and may be made using techniques known in the art, including *in vitro* transcription, nick translation, or kinase reactions. A nucleic acid sample containing a sequence capable of hybridizing to the labeled probe is contacted with the labeled probe. If the nucleic acid in the sample is double stranded, it may be denatured prior to contacting the probe. In some applications, the nucleic acid sample may be immobilized on a surface such as a nitrocellulose or nylon membrane. The nucleic acid sample may comprise nucleic acids obtained from a variety of sources, including genomic DNA, cDNA libraries, RNA, or tissue samples.

Procedures used to detect the presence of nucleic acids capable of hybridizing to the detectable probe include well known techniques such as Southern blotting, Northern blotting, dot blotting, colony hybridization, and plaque hybridization. In some applications, the nucleic acid capable of hybridizing to the labeled probe may be cloned into vectors such as expression vectors, sequencing vectors, or *in vitro* transcription vectors to facilitate the characterization and expression of the hybridizing nucleic acids in the sample. For example, such techniques may be used to isolate and clone sequences in a genomic library or cDNA library which are capable of hybridizing to the detectable probe as described in Example 30 above.

PCR primers made as described in Example 44 above may be used in forensic analyses, such as the DNA fingerprinting techniques described in Examples 46-50 below. Such analyses may utilize detectable probes or primers based on the sequences of the the 5' ESTs or of cDNAs or genomic DNAs isolated using the 5' ESTs.

(2) INFORMATION FOR SEQ ID NO: 297:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 224 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo Sapiens
(F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: 89..222
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 97
region 93..226
id W81645
est

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: 26..90
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 95
region 31..95
id W81645
est

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: 89..222
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 97
region 62..195
id W06951
est

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: 33..90
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 96
region 7..64
id W06951
est

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: 45..222
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 97
region 2..179
id W38711
est

(ix) FEATURE:
(A) NAME/KEY: sig_peptide

(B) LOCATION: 24..86
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 3.7
seq ETCALASHSGSSG/SK

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 297:

```
GCNGTCGGCT CCGCGGCGCC GCC ATG GCC GAC GTG GAA GAC GGA GAG GAA ACC   53
                          Met Ala Asp Val Glu Asp Gly Glu Glu Thr
                             -20                 -15

TGC GCC CTG GCC TCT CAC TCC GGG AGC TCA GGC TCC AAG TCG GGA GGC   101
Cys Ala Leu Ala Ser His Ser Gly Ser Ser Gly Ser Lys Ser Gly Gly
    -10                  -5                   1                5

GAC AAG ATG TTC TCC CTC AAG AAG TGG AAC GCG GTG GCC ATG TGG AGC   149
Asp Lys Met Phe Ser Leu Lys Lys Trp Asn Ala Val Ala Met Trp Ser
                10                  15                  20

TGG GAC GTG GAG TGC GAT ACG TGC GCC ATC TGC AGG GTC CAG GTG ATG   197
Trp Asp Val Glu Cys Asp Thr Cys Ala Ile Cys Arg Val Gln Val Met
            25                  30                  35

GAT GCC TGT MTT AGA TGT CAA GCG GGG                              224
Asp Ala Cys Xaa Arg Cys Gln Ala Gly
        40                  45
```

(2) INFORMATION FOR SEQ ID NO: 298:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 356 base pairs
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: DOUBLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: CDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo Sapiens
(D) DEVELOPMENTAL STAGE: Fetal
(F) TISSUE TYPE: kidney

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: 122..188
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 100
region 198..264
id R58050
est

(ix) FEATURE:
(A) NAME/KEY: other
(B) LOCATION: complement(122..188)
(C) IDENTIFICATION METHOD: blastn
(D) OTHER INFORMATION: identity 100
region 193..259
id H98670

(B) LOCATION: -17..-1
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: . score 3.7
seq RTWCLACVEASPG/QP

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 564:

```
Met Xaa Cys Pro Arg Thr Trp Cys Leu Ala Cys Val Glu Ala Ser Pro
       -15                 -10                 -5

Gly Gln Pro Phe Leu Pro Pro Arg Pro Gly
     1               5
```

(2) INFORMATION FOR SEQ ID NO: 565:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: AMINO ACID
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo Sapiens
(F) TISSUE TYPE: Dystrophic muscle

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: -21..-1
(C) IDENTIFICATION METHOD: Von Heijne matrix
(D) OTHER INFORMATION: score 3.7
seq ETCALASHSGSSG/SK

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 565:

```
Met Ala Asp Val Glu Asp Gly Glu Glu Thr Cys Ala Leu Ala Ser His
    -20                 -15                 -10

Ser Gly Ser Ser Gly Ser Lys Ser Gly Gly Asp Lys Met Phe Ser Leu
 -5                  1                   5                  10

Lys Lys Trp Asn Ala Val Ala Met Trp Ser Trp Asp Val Glu Cys Asp
             15                  20                  25

Thr Cys Ala Ile Cys Arg Val Gln Val Met Asp Ala Cys Xaa Arg Cys
         30                  35                  40

Gln Ala Gly
     45
```

(2) INFORMATION FOR SEQ ID NO: 566:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: AMINO ACID

CLAIMS

1. A purified or isolated nucleic acid comprising the sequence of one of SEQ ID NOs: 38-305 or comprising a sequence complementary thereto.

2. The nucleic acid of Claim 1, wherein said nucleic acid is recombinant.

3. A purified or isolated nucleic acid comprising at least 10 consecutive bases of the sequence of one of SEQ ID NOs: 38-305 or one of the sequences complementary thereto.

4. A purified or isolated nucleic acid comprising at least 15 consecutive bases of one of the sequences of SEQ ID NOs: 38-305 or one of the sequences complementary thereto.

5. The nucleic acid of Claim 4, wherein said nucleic acid is recombinant.

6. A purified or isolated nucleic acid of at least 15 bases capable of hybridizing under stringent conditions to the sequence of one of SEQ ID NOs: 38-305 or one of the sequences complementary to the sequences of SEQ ID NOs: 38-305.

7. The nucleic acid of Claim 6, wherein said nucleic acid is recombinant.

8. A purified or isolated nucleic acid encoding a human gene product, said human gene product having a sequence partially encoded by one of the sequences of SEQ ID NO: 38-305.

9. A purified or isolated nucleic acid having the sequence of one of SEQ ID NOs: 38-305 or having a sequence complementary thereto.

10. A purified or isolated nucleic acid comprising the nucleotides of one of SEQ ID NOs: 38-305 which encode a signal peptide.

11. A purified or isolated polypeptides comprising a signal peptide encoded by one of the sequences of SEQ ID NOs: 38-305.

12. A vector encoding a fusion protein comprising a polypeptide and a signal peptide, said vector comprising a first nucleic acid encoding a signal peptide encoded by one of the sequences of SEQ ID NOs: 38-305 operably linked to a second nucleic acid encoding a polypeptide.

13. A method of directing the extracellular secretion of a polypeptide or the insertion of a polypetide into the membrane comprising the steps of:

obtaining a vector according to Claim 12; and

introducing said vector into a host cell such that said fusion protein is secreted into the extracellular environment of said host cell or inserted into the membrane of said host cell.

14. A method of importing a polypeptide into a cell comprising contacting said cell with a fusion protein comprising a signal peptide encoded by one of the sequences of SEQ ID NOs: 38-305 operably linked to said polypeptide.

15. A method of making a cDNA encoding a human secretory protein that is partially encoded by one of SEQ ID NOs 38-305, comprising the steps of:

obtaining a cDNA comprising one of the sequences of SEQ ID NOs: 38-305;

contacting said cDNA with a detectable probe comprising at least 15 consecutive nucleotides of said sequence of SEQ ID NO: 38-305 or a sequence complementary thereto under conditions which permit said probe to hybridize to said cDNA;

identifying a cDNA which hybridizes to said detectable probe; and

isolating said cDNA which hybridizes to said probe.

16. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305 or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 15.

17. The cDNA of Claim 16 wherein said cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

18. A method of making a cDNA comprising one of the sequences of SEQ ID NOs: 38-305, comprising the steps of:

contacting a collection of mRNA molecules from human cells with a first primer capable of hybridizing to the polyA tail of said mRNA;

hybridizing said first primer to said polyA tail;

reverse transcribing said mRNA to make a first cDNA strand;

making a second cDNA strand complementary to said first cDNA strand using at least one primer comprising at least 15 nucleotides of one of the sequences of SEQ ID NOs 38-305; and

isolating the resulting cDNA comprising said first cDNA strand and said second cDNA strand.

19. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305 or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 18.

20. The cDNA of Claim 19 wherein said cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

21. The method of Claim 18, wherein the second cDNA strand is made by:

contacting said first cDNA strand with a first pair of primers, said first pair of primers comprising a second primer comprising at least 15 consecutive nucleotides of one of the sequences of SEQ ID NOs 38-305 and a third primer having a sequence therein which is included within the sequence of said first primer;

performing a first polymerase chain reaction with said first pair of nested primers to generate a first PCR product;

contacting said first PCR product with a second pair of primers, said second pair of primers comprising a fourth primer, said fourth primer comprising at least 15 consecutive nucleotides of said sequence of one of SEQ ID NO:s 38-305 , and a fifth primer, said fourth and fifth primers being capable of hybridizing to sequences within said first PCR product; and

performing a second polymerase chain reaction, thereby generating a second PCR product.

22. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein encoded by one of SEQ ID NOs 38-305, or a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 21.

23. The cDNA of Claim 22 wherein said cDNA comprises the full protein coding sequence partially included in one of the sequences of SEQ ID NOs: 38-305.

24. The method of Claim 18 wherein the second cDNA strand is made by:

contacting said first cDNA strand with a second primer comprising at least 15 consecutive nucleotides of the sequences of SEQ ID NOs: 38-305;

hybridizing said second primer to said first strand cDNA; and

extending said hybridized second primer to generate said second cDNA strand.

25. An isolated or purified cDNA encoding a human secretory protein, said human secretory protein comprising the protein partially encoded by one of SEQ ID NOs 38-305 or comprising a fragment thereof of at least 10 amino acids, said cDNA being obtainable by the method of Claim 24.

26. The cDNA of Claim 25, wherein said cDNA comprises the full protein coding sequence partially included in of one of the sequences of SEQ ID NOs: 38-305.

27. A method of making a protein comprising one of the sequences of SEQ ID NO: 306-573, comprising the steps of:

obtaining a cDNA encoding the full protein sequence partially included in one of the sequences of sequence of SEQ ID NO: 38-305;

inserting said cDNA in an expression vector such that said cDNA is operably linked to a promoter;

introducing said expression vector into a host cell whereby said host cell produces the protein encoded by said cDNA; and

isolating said protein.

28. An isolated protein obtainable by the method of Claim 27.

29. A method of obtaining a promoter DNA comprising the steps of:

obtaining DNAs located upstream of the nucleic acids of SEQ ID NO: 38-305 or the sequences complementary thereto;

screening said upstream DNAs to identify a promoter capable of directing transcription initiation; and

isolating said DNA comprising said identified promoter.

30. The method of Claim 29, wherein said obtaining step comprises chromosome walking from said nucleic acids of SEQ ID NO: 38-305 or sequences complementary thereto.

31. The method of Claim 30, wherein said screening step comprises inserting said upstream sequences into a promoter reporter vector.

32. The method of Claim 30, wherein said screening step comprises identifying motifs in said upstream DNAs which are transcription factor binding sites or transcription start sites.

33. An isolated promoter obtainable by the method of Claim 32.

34. An isolated or purified protein comprising one of the sequences of SEQ ID NO: 306-573.

35. In an array of discrete ESTs or fragments thereof of at least 15 nucleotides in length, the improvement comprising inclusion in said array of at least one of the sequences of SEQ ID NOs: 38-305, or one of the sequences complementary to the sequences of SEQ ID NOs: 38-305, or a fragment thereof of at least 15 consecutive nucleotides.

36. The array of Claim 35 including therein at least two of the sequences of SEQ ID NOs: 38-305, the sequences complementary to the sequences of SEQ ID NOs: 38-305, or fragments thereof of at least 15 consecutive nucleotides.

37. The array of Claim 35 including therein at least five of the sequences of SEQ ID NOs: 38-305, the sequences complementary to the sequences of SEQ ID NOs: 38-305, or fragments thereof of at least 15 consecutive nucleotides.